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Reference Ranges for Thyrotropin in the Serum of Full-Term Neonates – Compared with the Ranges for Full-Term Neonates with Various Post-Partal Adaptation Disorders, and Premature Neonates

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Summary: The "IMx hTSH ultrasensitive test" from Abbott Laboratories is a new method for the determination of thyrotropin. Its introduction for the purpose of hypothyreosis screening led to the present investigation of the reference ranges in full-term and premature neonates. In a total of 1712 healthy neonates, the reference range for serum thyrotropin on the 5th day post partum was 0.4–9.05 mU/l (median value 1.90 mU/l). In contrast, 64 full-term neonates with various post-partal adaptation disorders showed a significantly lower serum thyrotropin concentration on the 5th day post partum (0.14–6.39 mU/l; median value 1.60 mU/l). Serum thyrotropin was also determined on the 7th and 14th day post partum in 131 premature neonates with birth-weights below 2500 g. In the birth-weight range 1500– <2500 g, the median values for serum thyrotropin were 2.35 and 2.12 mU/l, respectively. A negative correlation ($r = -0.3019$) was found between the birth-weight and the serum thyrotropin concentration on the 14th day post partum. In two premature neonates with birth-weights less than 800 g, the serum thyrotropin concentrations were conspicuously high (18.6 and 28.0 mU/l) on the 14th day post partum.

Introduction

Since the introduction of neonatal screening for congenital hypothyreosis, the number of cases of mental retardation due to this condition has markedly decreased in most industrialized countries (1). It has been shown that congenitally hypothyreotic children, discovered by screening, display normal intellectual and physical development for their age, provided therapy is started as soon as possible within the first four weeks post partum with a sufficiently high dose of thyroxine (2–5).

In most European countries, hypothyreosis screening of neonates is performed by the determination of thyrotropin in small filter paper discs impregnated with whole blood obtained on the 5th day post partum. In most cases, a radioimmunoassay is used for the determination (1, 6–8). In the USA, however, thyrotropin is determined only if the same filter paper sample is found to contain an abnormally low concentration of thyroxine (1, 9).

In addition to technical problems associated with the collection and processing of samples for screening, physiological changes in the thyroid function of neonates, especially premature neonates, are responsible for a varying number of false positive and very small number of false negative results (1, 6, 9).

The aim of the investigation was to use the "IMx hTSH ultrasensitive test" from Abbott Laboratories to establish *reliable* reference ranges for thyrotropin in the serum of healthy neonates on the 5th day post-partum and premature neonates on the 7th and 14th day post partum, and to determine whether the results from these groups are significantly different.

Materials and Methods

All full-term and premature neonates born in Erfurt between November 1990 and January 1992 were included in the investigation (tab. 1). The resulting collective consisted of 1712 healthy full-term neonates (866 male, 846 female), 64 full-term neonates with post-partal adaptation disorders (40 male, 24 female), and 131 neonates with a birth weight below 2500 g (61 male, 70 female).

Tab. 1. Structure of the proband collective

Group	n	Birth-weight (g)	
1	1712	>2500	Full-term healthy neonates
2	64	>2500	Full-term healthy neonates with post-partal adaptation disorders
3	83	1500 – <2500	Premature neonates
4	36	1000 – <1500	Premature neonates
5	12	<1000	Premature neonates

Sample material

Blood (0.5 – 1.0 ml) was taken from a peripheral vein of each of the 1907 neonates, on the 5th, 7th and/or 14th day post partum, using a brown microvette (Sarstedt). Serum was prepared within 1 hour by centrifugation, then analysed immediately or stored at −20 °C for no longer than 48 hours before analysis.

Procedure

Thyrotropin was determined with the "IMX hTSH ultrasensitive test" from Abbott Laboratories (sensitivity according to the manufacturer: 0.03 mU/l). This test is a microparticle-enzyme-immunoassay (MEIA).

Quality control

To monitor the accuracy and precision from day to day, control samples of low, medium and high concentrations from Abbott and from Greiner were analysed intermittently in each series. As a measure of the relative error of the method, the arithmetic mean ($\bar{x}$), standard deviation (s), and the variation coefficient (CV) were calculated from the results of the control sera.

Statistical evaluation of results

Results were evaluated statistically with the SPSS program. None of the groups showed a normal *Gauss*ian distribution (fig. 1). Therefore the 2.5 and 97.5 percentiles were calculated for the 95% scatter range, together with the median value (50 percentile) and the 5 and 95 percentiles. The U-test of *Mann* and *Whitney* was used to test for significant differences between the groups. For the premature neonates with a birth-weight below 2500 g, the degree of linearity between gestation time, birth-weight and serum thyrotropin on the 7th and 14th day post partum was investigated by determination of the correlation coefficient (r).

Results

Serum thyrotropin concentrations in 1712 healthy neonates with a birth-weight >2500 g were in the range 0.40 – 9.05 mU/l (95% scatter range) (tab. 2). In contrast, 64 full-term neonates with various post-partal adaptation disorders showed significantly lower serum thyrotropin concentrations, i.e. 0.16 – 6.39 mU/l (95% scatter range) (tab. 2).

In the premature neonates with a birth-weight of 1500 – <2500 g, the median serum thyrotropin concentrations were 2.35 and 2.12 mU/l on the 7th and 14th day post partum, respectively, i.e. they were higher than in full-term neonates (tab. 2). The differences, however, were statistically significant only with respect to the full-term neonates with post-partal adaptation disorders (tab. 3).

Elevated median serum thyrotropin values were also found in the group of premature neonates with a birth-weight of 1000 – <1500 g i.e. 2.55 and 2.67 mU/l on the 7th and 14th day post partum, respectively. Owing to the small number of probands, only the 90% scatter range, rather than the 95% range, could be established (tab. 2). On the 7th day post partum, the values for this group were conspicuously higher than those for both groups of full-term neonates (tab. 2, tab. 3, fig. 1c). On the 14th day post partum, the values for this group differed significantly from those for group 1 and group 3b (tab. 3).

Tab. 2. Summary of the results of the thyrotropin determinations (mU/l).
Group 1: full-term healthy neonates with a birth-weight >2500 g.
Group 2: full-term neonates with post-partal adaptation disorders, birth-weight >2500 g.
Groups 3a and 3b: premature neonates, birth-weight 1500 – <2500 g.
Groups 4a und 4b: premature neonates, birth-weight 1000 – <1500 g.
Groups 5a and 5b: premature neonates, birth-weight <1000 g.

Group	Day post partum	n	Minimum	Percentiles					Maximum
				2.5	5	50	95	97.5	
1	5	1712	<0.03*	0.40	0.53	1.90	6.73	9.05	22.0
2	5	64	<0.03*	0.16	0.33	1.60	5.18	6.39	7.51
3a	7	72	0.51	0.58	0.64	2.35	6.29	7.86	9.57
3b	14	74	0.20	0.59	0.69	2.12	4.24	4.97	5.26
4a	7	24	0.64	–	0.87	2.55	11.9	–	13.7
4b	14	30	0.88	–	0.93	2.67	10.6	–	14.0
5a	7	4	1.20	–	–	2.41	–	–	3.79
5b	14	11	1.11	–	–	3.73	–	–	28.0

* These concentrations occurred only once.

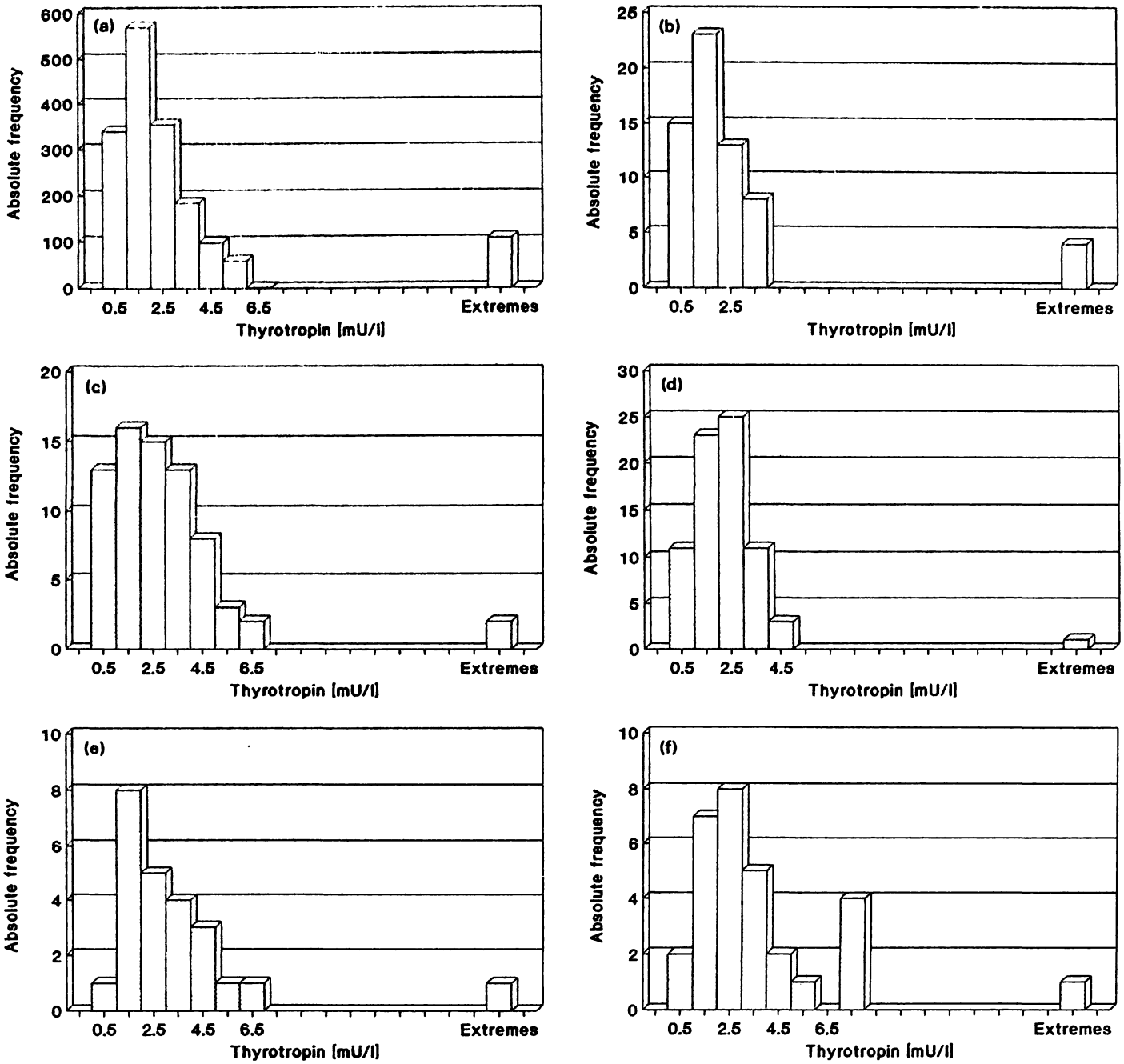

Fig. 1. Histograms of the results of serum thyrotropin determinations (mU/l) performed
a) on the 5th day post partum in full term healthy neonates, birth-weight >2500 g (group 1); n = 1712;
b) on the 5th day post partum in full-term neonates with post-partal adaptation disorders, birth-weight >2500 g (group 2); n = 64;
c) on the 7th day post partum in premature neonates, birth-weight 1500– <2500 g (group 3a); n = 72;
d) on the 14th day post partum in premature neonates, birthweight 1500– <2500 g (group 3b); n = 74;
e) on the 7th day post partum in premature neonates, birth-weight 1000– <1500 g (group 4a); n = 24;
f) on the 14th day post partum in premature neonates, birth-weight 1000– <1500 g (group 4b); n = 30.

Tab. 3. Probabilities (p) that the conditions of the null hypothesis are met, as calculated by the U-test of *Mann* and *Whitney*. For two-sided significance limits and an error probability of $\alpha = 0.05$, the difference between two groups is taken as significant, if $p < 0.05$. Significant differences are indicated by *.

Group	2	3a	3b	4a	4b
1	0.0248*	0.0957	0.7960	0.0133*	0.0030*
2		0.0066*	0.0248*	0.0009*	0.0618*
3a				0.2167	
3b					0.0078*

Table 4 shows the individual results for thyrotropin in the serum of neonates with a birth-weight below 1000 g. Owing to the small number of cases, the calculation of scatter ranges was inappropriate. On the 7th day post partum, serum could be obtained from only four of these premature neonates. At this sampling time, the results were not noticeably different from those of other groups.

On the 14th day post partum, the serum thyrotropin concentrations of three of these probands had

Tab. 4. Individual results of the serum thyrotropin determinations in premature neonates with a birth-weight < 1000 g on the 7th and 14th days post partum.

	Sex	Gestation time (weeks)	Birth-weight (g)	Thyrotropin	
				7th day (mU/l)	14th day (mU/l)
1	♂	30	840	1.20	
2	♂	28	880	2.41	2.58
3	♂	28	960		5.18
4	♀	28	815	3.79	3.73
5	♂	29	870	2.41	2.93
6	♀	28	885		5.04
7	♀	27	860		2.77
8	♀	27	790		28.0
9	♂	28	990		3.36
10	♀	31	850		7.37
11	♂	26	820		1.11
12	♀	26	700		18.6

changed only insignificantly. It is striking, however, that the premature neonates, in particular those with birth-weights below 800 g, showed markedly elevated serum thyrotropin concentrations. As shown in table 2, the median value for serum thyrotropin for the entire group was 3.73 mU/l on the 14th day, and therefore markedly higher than in all other groups. In view of the small number of cases, however, a test of significance did not appear justified.

Analysis of the correlation between the birth-weight of all premature neonates and their serum thyrotropin concentrations on the 7th and 14th day post partum showed a negative correlation ($r = -0.3019$) on the 14th day, i. e. the thyrotropin concentrations increased with decreasing birth-weight. On the other hand, no correlation was found between gestation time and serum thyrotropin concentration ($r = -0.0689$ and -0.1322, respectively).

Results from the control of precision and accuracy of the thyrotropin determinations are summarized in table 5. As a measure of precision, the day-to-day variation coefficient for the analysis of control sera was between 4.44 and 9.74%. Variation coefficients for serial analyses of the control sera, Serodos and Serodos Plus, are given in table 6.

Tab. 5. Results for the control of precision and accuracy from day to day.

Control serum	n	$\bar{x}$ (mU/l)	s	CV (%)
Serodos (Greiner)	19	1.00	0.08	8.00
Serodos Plus (Greiner)	31	13.97	0.76	5.44
Abbott M ($\bar{x}$ = 6.00 mU/l)	56	5.84	0.26	4.45
Abbott H ($\bar{x}$ = 50.0 mU/l)	49	49.15	4.79	9.74

Tab. 6. Results for the control of precision in series.

Control serum	n	$\bar{x}$ (mU/l)	s	CV (%)
Serodos (Greiner)	22	1.05	0.04	3.72
Serodos Plus (Greiner)	22	34.47	1.07	3.11

Discussion

Serum thyrotropin concentrations were determined in 1776 full-term neonates on the 5th day post partum, to determine the reference ranges that are necessary for the recognition of congenital hypothyreosis. Of these 1776 neonates, 64 were eliminated on the basis of a variety of postnatal disorders. The measured serum thyrotropin values of the remaining 1712 neonates showed a median value of 1.90 mU/l for a 95% scatter range of 0.40–9.05 mU/l. The values for the 64 full-term neonates with adaptation disorders were significantly lower, with a median value of 1.60 mU/l and a 95% scatter range of 0.16–6.39 mU/l.

In premature neonates, thyrotropin was determined on the 7th and 14th day post partum, in accordance with the recommendations of the working group "hypothyreosis screening" of the former German Democratic Republic (10). Unfortunately, not all the premature neonates could be included. In the group with a birth-weight of 1500–<2500 g, the median values on the 7th and 14th days post partum were higher than in the full-term neonates, but the 95% scatter range was not significantly higher. In the 30 neonates with a birth-weight of 1000–<1500 g, only the 90% scatter range could be calculated, owing to the small size of the group. The results, however, were significantly higher than those for the full-term neonates.

On account of their small number, the premature neonates with a birth-weight below 1000 g could not be statistically evaluated. Two extremely small premature neonates weighing less than 800 g had serum thyrotropin concentrations of 18.6 and 28.0 mU/l. These two premature babies were in an artificial respirator, and they were fed entirely parenterally, but on the 14th day post partum they showed no postnatal complications and no apparent deformities.

Direct comparison of the present results with those of other authors in not possible, due to the use of different analytical methods and different sampling times (11, 12). Whereas earlier studies (13–15) described a positive correlation between gestation time and serum thyrotropin concentration, this was not found in the present investigation. There was a negative correlation between birth-weight and serum thyrotropin concentration on the 14th day post partum.

The results of the present study contribute to the discussion of whether elevated serum thyrotropin concentrations in premature neonates are normal, or whether they are pathological and require treatment. This question can only be resolved by a combined determination of thyrotropin, triiodothyronine, thyroxine, free thyroxine and reverse triiodothyronine during the first 14 days post partum. The aim of this investigation, however, was to determine reference ranges for normal values of serum thyrotropin. In the view of the authors, the volume of serum (50–100 µl) required for hypothyreosis screening of neonates is justifiable. This volume enables the simple, rapid, safe and controlled determination of serum thyrotropin.

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